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Fine mapping a quantitative trait locus affecting ovulation rate in swine on chromosome 8[1]

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ABSTRACT: Ovulation rate is an integral component of litter size in swine, but is difficult to directly select for in commercial swine production. Because a QTL has been detected for ovulation rate at the terminal end of chromosome 8p, genetic markers for this QTL would enable direct selection for ovulation rate in both males and females. Eleven genes from human chromosome 4p16-p15, as well as one physiological candidate gene, were genetically mapped in the pig. Large insert swine genomic libraries were screened, clones were isolated and then screened for microsatellite repeats, and informative microsatellite markers were developed for seven genes (GNRHR, IDUA, MAN2B2, MSX1, PDE6B, PPP2R2C, and RGS12). Three genes (LRPAP1, GPRK2L, and FLJ20425) were mapped using genotyping assays developed from single nucleotide polymorphisms. Two genes were assigned since they were present in clones that contained mapped markers (HGFAC and HMX1). The resulting linkage map of pig chromosome 8 contains markers associated with 14 genes in the first 27 cM. One inversion spanning at least 3 Mb in the human genome was detected; all other differences could be explained by resolution of mapping techniques used. Fourteen of the most informative microsatellite markers in the first 27 cM of the map were genotyped across the entire MARC swine resource population, increasing the number of markers typed from 2 to 14 and more than doubling the number of genotyped animals with ovulation rate data (295 to 600). Results from the revised data set for the QTL analysis, assuming breed specific QTL alleles, indicated that the most likely position of the QTL resided at 4.85 cM on the new linkage map ($F_{1,592}$ = 20.5150, genome-wide probability less than 0.015). The updated estimate of the effect of an allele substitution was –1.65 ova for the Meishan allele. The *F*-ratio peak was closest to markers for MAN2B2 (4.80 cM) and was flanked on the other side by markers for PPP2R2C. Two positional candidate genes included in this study are MAN2B2 and RGS12. These results validate the presence of a QTL affecting ovulation rate on chromosome 8 and facilitate selection of positional candidate genes to be evaluated.

Key Words: Litter Size, Ovulation Rate, Quantitative Trait Loci, Pigs

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Introduction

Because litter size is extremely important to the swine industry, it would be advantageous for swine producers to be able to select replacement gilts that had the potential to have larger litters than their peers. Although the heritability for litter size is low (Lamberson, 1990), it has been proposed that a greater response in litter size could be achieved by selecting for increased ovulation rate and increased uterine capacity (Bennett and Leymaster, 1989). However, neither of these traits can be easily measured. Selection for ovulation rate and uterine capacity could be facilitated by the identification of genetic markers associated with DNA variants affecting these traits.

Rohrer et al. (1999) reported a QTL for ovulation rate on the p arm of porcine chromosome 8 (**SSC8**), along with a QTL affecting plasma FSH in pubertal boars (Rohrer et al., 2001). Porcine chromosome 8 is orthologous to human chromosome 4 based on bidirectional fluorescent in situ hybridization (Goureau et al., 1996). Rohrer (1999) mapped the gene PDE6B, which resides at HSA4p16.3, to the region where the QTL for ovulation rate exists at SSC8p2.3. In order to further characterize the area surrounding these QTL and to increase the precision of the estimates for the ovulation rate QTL

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Table 1. Data for genes known or predicted to map to SSC8

Gene acronym	Gene name	Location, Mb[a]
FLJ20425	Hypothetical protein	4.2
GPRK2L	G-protein coupled receptor kinase 2-like	3.0
GNRHR	Gonadotropin releasing hormone receptor	68.5
HGFAC	Hepatocyte growth factor activator	3.4
HMX1	Homeobox (H6 family) 1	21.2
IDUA	α-L-iduronidase	1.0
LRPAP1	Low-density lipoprotein-related protein-associated protein 1	3.5
MAN2B2	α-Mannosidase 2, B2 (also KIAA 0935)	6.5
MSX1	msh homeobox homolog 1 (formerly HOX7)	4.7
PDE6B	Phosphodiesterase 6B	0.6
PPP2R2C	Protein phosphatase 2, regulatory subunit B, γ isoform	6.3
RGS12	Regulator of G-protein signaling 12	3.3

[a]Position was determined from the June 2002 build of the human genome as presented on the GoldenPath viewer (http://genome.ucsc.edu/).

parameters, genes located on HSA4p16 were mapped in the porcine genome and the most highly informative markers from the updated map were genotyped across animals of the original analysis (Rohrer et al., 1999), as well as 305 additional F_3 gilts.

Materials and Methods

A total of 12 genes located on HSA 4 were studied. The selected genes, their acronym, and location in the human genome are presented in Table 1. Eleven of the genes were selected based on their assignment to HSA 4p16 or 4p15 (base positions of 0 to 11.7 Mb for 4p16, 11.7 to 37.3 Mb for 4p15) to determine the boundaries of the ovulation rate QTL on the human genome map, as well as to study the conservation of gene order. A genetic marker for GNRH receptor (**GNRHR**; Table 1) was developed because a yeast artificial chromosome (**YAC**) clone was available from our previous study (Rohrer, 1999). To increase marker density for this region, a YAC clone that contained S0098 and cosmid clones that yielded SW2410 and SW2611 were screened for additional microsatellites.

Microsatellite Marker Development and Genotyping

Microsatellite (CA/GT) repetitive elements were isolated from large swine genomic clones determined to contain genes of interest. Yeast artificial chromosome clones were identified by PCR using pooled DNA from the porcine YAC library described by Alexander et al. (1997), whereas cosmid clones were identified from a purchased porcine cosmid library by an iterative PCR technique (Smith et al., 1995). The bacterial artificial chromosome (**BAC**) clones were isolated from the Roswell Park Cancer Institute (Buffalo, NY) (RPCI)-44 porcine BAC library by hybridization with 1×10^6 counts/filter of each [α^{32}P] dATP-labeled probe. Probes were generated either by labeling PCR amplicons with the MegaPrime DNA Labeling System (Amersham Pharmacia Biotech, Piscataway, NJ) or direct incorporation of radioisotope, and were then cleaned with a GS-25 sephadex column (5Prime3′, Boulder, CO). Positive clones were grown overnight, and DNA was extracted using a Qiagen miniprep kit (Qiagen, Valencia, CA). For BAC DNA, the Qiagen procedure was modified by adding overnight room temperature incubation after the elution of the BAC DNA from the columns and the addition of isopropanol before centrifugation.

Microsatellite repeats were identified by digestion of the BAC, YAC, or cosmid clone DNA with either *Tsp*509I or *Sau*3AI and were ligated into *Eco*RI or *Bam*HI digested pBluescript transformed into XL1 BLU *Escherichia coli* (Stratagene, La Jolla, CA) plated out and grown overnight. Colony lifts were probed with [γ^{32}P] dATP kinased $(GT)_{11}$, four or five positive colonies (when possible) were grown in 5 mL of Luria-Bertani medium with 50 μg/mL of ampicillin overnight, and then DNA was prepared using QIAprep miniprep kits (Qiagen). The sequencing reactions were performed with 2 μL of prepared plasmid DNA, 1 μL of 3.2 μ*M* M13 primer, and 2 μL of ABI Big Dye (Perkin Elmer Corp., Foster City, CA) and run on an ABI-377 (Perkin Elmer Corp.). Sequences were deposited into GenBank. Primers were designed to amplify the region containing the GT repeat (Table 2).

Microsatellite markers were genotyped in the USDA, ARS, U.S. Meat Animal Research Center's (MARC) swine reference population (Rohrer et al., 1994) and linkage analysis was performed with all SSC 8 genetic markers located in the MARC genome database using CRI-MAP (version 2.4; Green et al., 1990). Once the final marker order was determined, the CHROMPIC option of CRI-MAP was implemented to determine suspect genotypes. All suspect genotypes were evaluated and corrections were made when necessary. Selected microsatellite markers were also genotyped on the MARC swine resource population—which is comprised of a Meishan and White Composite cross—using the same methods (Rohrer et al., 1999).

Single Nucleotide Polymorphism Marker Development and Genotyping

Single nucleotide polymorphism (**SNP**) markers were developed for G-protein coupled receptor kinase 2-like

Table 2. New microsatellite markers developed from genomic clones

Marker	Gene probe[a]	Primers	Annealing temperature, °C	Number alleles	Informative meioses	Product size, bp
PDE6B	PDE6B[C]	caagagtcgctgcagaag catacccсttcacacacgc	60	4	79	135 to 143
MANMS	MAN2B2[C]	agacctgcaacctgcagtg tgaatgggaaagggagagg	58	5[b]	62	181 to 211
SW2651	SW2410[C]	tggctcagaggctgtcaac actgtttacacaggcagggg	58	3	116	100 to 106
SW2652	SW2611[C]	ctccctttccagcccttc atacaagcaatgtagcaatgttcc	58	6	137	235 to 297
SY12	IDUA[Y]	tcggattctttcctcctgtg tcacttgtgatggaacatgatg	58	4	64	185 to 224
SY13	IDUA[Y]	tgcaaagttaccacacggac cgtgcatgacatacctctgg	58	2	47	118 to 122
SY14	GNRHR[Y]	cagagatgtttgaatgtgtctgc gctcagtttccatattgtgc	58	3	108	106 to 112
SY20	MSX1[Y]	tgcctgctctactaccagagtg agttgagaacctcagtgcagtg	55	6	65	133 to 155
SY21	S0098[Y]	tcaggaccagcttgaatgc agttgagaacctcagtgcagtg	55	4	79	178 to 195
SY22	S0098[Y]	gctagagatggagaggcgg gtatgggtacgcacatgagc	55	4	123	157 to 165
SY23	MSX1[Y]	aactatgacggccgctactg ccccataatttcaccagcag	55	6	127	90 to 124
SY27	MSX1[Y]	ccaggttgtagcaaatggtg agcccctcaacagatgaatg	60	5	138	132 to 156
SY30	MAN2B2[Y]	tgtgttagtttcaggtggatgg ccaaggacctactgcagagc	58	3	46	174 to 192
SY35	MSX1[Y]	tgtcagtaacaatggctatggc ggaaagcaagtgtgggagag	58	6	85	120 to 146
SY36	MSX1[Y]	gtctcacatcaaggctcaaatg agaaagatagctgcaggtgtcc	58	3	77	206 to 220
SB37	MAN2B2[B]	gaatggataagcagtgaggtcc ttgagtggcacctgtttttg	55	6	77	248 to 288
SB60	PPP2R2C[B]	ttctgggtggacacaacattc atttgtcgtggctgtgtgc	58	3	136	223 to 231
SB62	PPP2R2C[B]	tcgctggaatatttatttattagga accactggagtaccacgaag	55	6	145	126 to 139
SB73	RGS12[B]	ctcacgcttcctcacttagca acgggtttctgtggagttttt	55	6	158	240 to 262
SB74	RGS12[B]	tcacggctcttgatggcacag tctttcccctgtatctcccac	58	3	115	164 to 190

[a]Type of genomic clone is represented by a superscript letter. B = BAC clone, C = cosmid clone, and Y = YAC clone. See Table 1 for gene names.
[b]Number of alleles includes a null allele.

(**GPRK2L**) and a hypothetical protein designated as FLJ20425. Amplicons were designed to amplify across an intron or 3′ untranslated regions; PCR was conducted on the parents of the MARC reference family and the products were sequenced; SNP was tagged as described by Fahrenkrug et al. (2002), and the sequences were submitted to GenBank. Genotyping assays were designed for the most informative SNP within each amplicon.

The MassArray Genolyzer (Sequenom Inc., San Diego, CA) system was used for SNP genotyping. Assays were based on the addition of one or two nucleotides to an oligonucleotide primer adjacent to the polymorphic base. Extended products were separated with a time-of-flight mass spectrometer (Bruker Biflex III Linear Time of Flight Mass Spectrometer; Bruker Daltonics, Bremen, Germany) and genotypes were called.

QTL Data and Analysis

The swine population was described in Rohrer et al. (1999). Briefly, White Composite (a four-breed composite developed at MARC) and Meishan animals were mated to produce F_1 females. The F_1 females were mated to parental breed sires to produce both backcross genotypes (**BC** generation); BC animals were reciprocally mated to produce ½ Meishan, ½ White composite animals in the F_3 generation, and F_3 animals were *inter se*-mated to produce the F_4 generation. Rohrer et al. (1999) used all females with phenotypic measurements in the BC and F_4 generations, but only 25% of the females in the F_3 generation. For this study, all females with phenotypic measurements for ovulation rate were used, which included 101 BC, 389 F_3, and 110 F_4 females.

The additional 305 F_3 females were genotyped for the markers used for the genome scan (SW2611 and SW1117). Based on the total number of alleles and the distribution of alleles within each breed, 12 additional microsatellite makers were genotyped across the entire population. These 14 markers spanned 27 cM. Marker density was greatest in the area where the original QTL was detected (nine markers located in the first 7 cM of the linkage group).

Statistical analyses were conducted using the same model described by Rohrer et al. (1999). Regression coefficients for the probability that an allele originated from the Meishan breed (Haley et al., 1994) were used. All four genotypic combinations were initially evaluated, and genotypic effects were removed as described by Rohrer et al. (1999). Fixed effects included in the model were contemporary group and breed composition. Nominal and genome-wide significance values are reported (Lander and Kruglyak, 1995).

Results

New Marker Development

Clones from the BAC, YAC, and cosmid libraries were identified, which contained α-mannosidase 2B2 (**MAN2B2**). Informative microsatellite markers were developed from all vectors; however, all microsatellites that mapped to SSC 8p were derived from the cosmid and BAC clones. Clones from the BAC library were identified that contained protein phosphatase 2 regulatory subunit B γ-isoform (**PPP2R2C**), regulator of G-protein signaling 12 (**RGS12**), and one clone that contained both low-density lipoprotein-related protein-associated protein 1 (**LRPAP1**) and hepatocyte growth factor activator (**HGFAC**). Clones from the YAC library were identified for homeo box 7 (**MSX1**), S0098, α-L-iduronidase (**IDUA**), and GNRHR (Rohrer, 1999). The cosmids for phosphodiesterase 6B (**PDE6B**; Rohrer, 1999), SW2410, and SW2611 (Alexander et al., 1996) previously identified were included.

A total of 13 microsatellite markers were added to SSC 8 from these clones. The number of alleles and number of informative meioses in the MARC reference population for the informative microsatellite markers are presented in Table 2. Only one marker was developed from each of the cosmid clones. The number of microsatellite markers successfully developed from each BAC and YAC clone ranged from one to five, depending on the quality of sequence obtained and the number of unique subclones sequenced. Three out of the five selected YAC clones were chimeric since SY12 from the IDUA YAC mapped to SSC 5q, SY30 from the MAN2B2 YAC mapped to SSC 10q, and microsatellite markers SY20, SY27, SY35, and SY36 from the MSX1 YAC all mapped to the centromeric region of SSC 4 (for specific locations, see http://www.marc.usda.gov/). No informative microsatellite markers were developed from the BAC containing LRPAP1, HGFAC and a portion of RGS12.

Table 3 presents information for the SNP markers developed and the number of informative meioses in the MARC reference population. Assays were designed for SNP associated with three genes.

The primers designed for H6 homeo box 1 (**HMX1**) did not give a single product from genomic DNA. However, it was determined that HMX1 was present in the cosmid that contained SW2410. A single PCR product was obtained from the cosmid and sequencing of the amplicon verified that it was HMX1. Likewise, no markers were developed specifically for HGFAC. Because HGFAC was present in a BAC, which also contained LRPAP1 and a portion of RGS12, its location on the swine genetic map can be inferred.

The updated linkage map for the MARC swine reference population is presented in Figure 1, along with the map derived from the resource population using selected microsatellite markers. As expected, the marker order is the same in both populations and the estimated interval sizes were comparable. The least robust marker was SWC31. This marker has only two alleles and is not very informative in any population studied at MARC. Its position from the analysis of the MARC reference population was based on only 26 informative meioses, but there were 244 informative meioses in the resource population.

Updated QTL Analysis

Using 600 animals with both phenotypic and genotypic data, the maximal F-ratio was $F_{1,592} = 20.5150$ at position 4.85 cM (Figure 2). The approximate one log-likelihood support confidence interval is 2.6 to 9.5 cM. The statistical model tested for the presence of a QTL at 0.05-cM intervals. As previously shown, F-ratios decrease sharply when the QTL is positioned directly on top of a genetic marker. Therefore, the F-ratios for analyses where the QTL was positioned over a genetic marker were removed for the plot presented in Figure 2. The peak position is 0.05 cM after marker SB37 (from the MAN2B2 BAC) and 0.25 cM before marker SB62 (from the PPP2R2C BAC). The mode of inheritance remained purely additive, but the magnitude of the effect was much lower than previously reported. Nonetheless, the QTL peak was still significant at the ge-

Table 3. Single nucleotide polymorphism markers developed

Marker	Gene	Sequence[a]	Location	Informative meioses
SNP13033	GPRK2L	GCCCTGACGC[A/G]TGCCTGCTGC	Intron 14	84
SNP16873	FLJ20425	CCTTGAAGTT[A/G]CTAAAGGATA CTTCCTTTCA[C/T]GACTGCTTGT	3′UTR	124
SNP21953	LRPAP1	GGCACCAGCG[A/G]GCTGGGGTTC CAGTGGAGGC[A/G]TGACGGGGAC GGACCAGCCA[C/T]GGGGGCCCGG	3′UTR	65

[a]Complete sequence of each amplicon can be obtained from GenBank, accession numbers: AF526390 (FLJ20425), AF526391 (GPRK2L), and AF526393 (LRPAP1). See Table 1 for gene names.

nome-wide level of significance. The estimated effect for each Meishan allele was –1.65 ova, indicating that on average, a female homozygous for the Meishan allele ovulated 3.3 fewer ova than a female homozygous for the White Composite allele.

Discussion

In this study, genetic markers were developed for genes located on human chromosome 4. The SNP markers all reside within the unprocessed RNA molecule transcribed for these genes. However, the location of the microsatellite markers relative to the gene is unknown. For microsatellite markers developed from cosmid clones, the marker is probably no more than 30 kb from the gene. Bacterial artificial chromosome clones can be as large as 200 kb and YAC clones can be as big as 1.5 Mb. Therefore, microsatellite markers developed from BAC and YAC clones may be closer to genes other than the target gene. In fact, the sequence that contained SY14 from a YAC for GNRHR also contained all of exon 2 from the UDP glucosyl transferase 1, polypeptide B15 (**UGT2B15**) gene, indicating SY14 is in intron 1 of this gene. Since UGT2B15 and GNRHR are 0.8 Mb apart in the human genome and the YAC was estimated to be 1.1 Mb, these results indicate that the distance in the pig genome could be similar.

Twelve genes were added to the MARC SSC 8 linkage map, 11 of which map within a region that contains a QTL for ovulation rate. Figure 3 depicts the comparative map for the region surrounding this QTL region. Three additional genes recently mapped and included in Figure 3 are Wolframin syndrome 1 (Rohrer et al., 2002), amino peptidases (Smith et al., 2001), and Huntington's disease (**HD**; Matsuyama, et al., 2000). One inversion was detected between HSA 4pter-p15 and SSC 8p2.3. The inversion involves five genes separated by 6 cM on the swine genetic map and 3 Mb on the human physical map (based on the June 2002 build displayed at http://genome.ucsc.edu/).

The only other major difference in gene order between human and pig maps was the location of HMX1. One other difference identified in the gene order, between GPRK2L and HD, was quite small, less than 2 cM on the swine genetic map and a few hundred kilobases on the human genomic sequence. This particular region of the human genome has changed considerably between the August 2001, December 2001, April 2002, and June 2002 builds. The entire region between 4 and 10 Mb has flipped in each subsequent update from August 2001 and April 2002. The HMX1 was removed from the August 2001 map in later builds until it was added in June 2002. Therefore, it is possible that the discrepancy in HMX1 will be resolved in later builds of the human genome data.

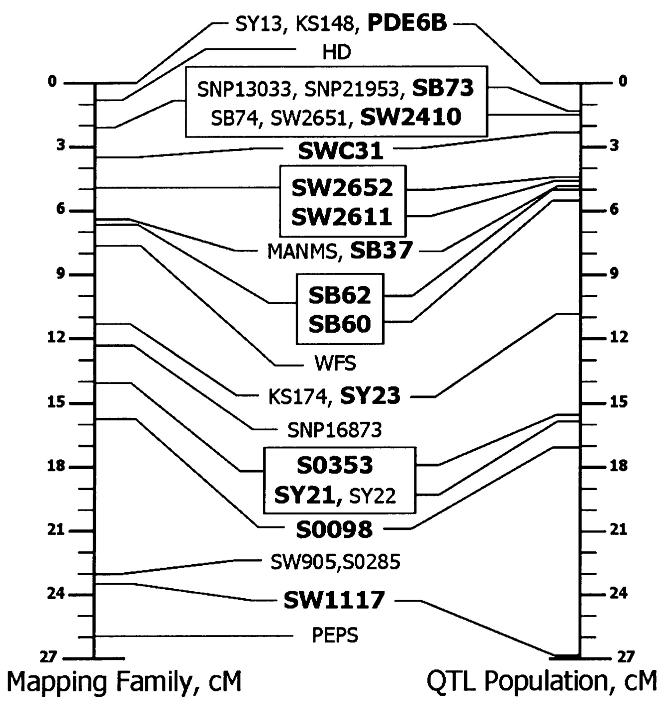

Figure 1. Genetic linkage map of the first 27 cM of porcine chromosome 8 (SSC) representing SSC 8p2.3. The map on the right is based on the MARC swine reference population described by Rohrer et al. (1994), which has been used as the base mapping family, and results are presented on the Web at http://www.marc.usda.gov/. Markers displayed in bold were mapped in the QTL mapping families (resource population) as described in the text, and the diagram on the left indicates the relative positions for this population. Markers located on the same line or within a box were at the same genetic position in the MARC reference population (mapping family).

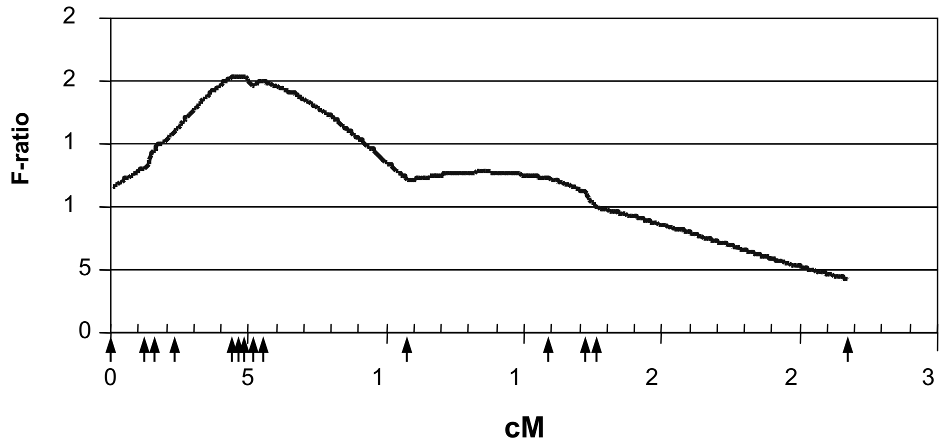

Figure 2. A summary of *F*-ratios (y-axis) computed every 0.05 cM (x-axis) for a QTL affecting the number of ova ovulated. The *F*-ratio had 1 df for the numerator fitting an additive genetic effect and 592 df for the error term. Arrows on the x-axis indicate positions where a genetic marker was typed in the QTL population. The markers and precise locations are presented in Figure 1. Threshold for genome-wide significance (0.05) was $F = 17.23$, and the pseudo one log-likelihood drop confidence interval is between 2.6 and 9.5 cM.

Jiang et al. (2002) has placed HD, GPRK2L, SW2410, MSX1, and SW1117 on a radiation hybrid panel and obtained the same order for these markers as that found in the present study. Our position of MAN2B2 on the porcine genetic map agrees with its physical assignment to SSC 8p2.3 (Ohata et al., 1997).

The results of Lahbib-Mansais et al. (1999) do not agree with the current study since they assigned fibroblast growth factor receptor 3 (**FGFR3**) to 8p11 using a somatic cell hybrid panel. The corresponding region to SSC 8p1.1 on the linkage map would be position 60 to 65 cM. From the location of FGFR3 on the human genome sequence (1.1 Mb), it should map to the interval between IDUA and GPRK2L located at SSC 8p2.3. Unfortunately, primers able to consistently amplify FGFR3 in porcine genomic DNA were not developed in the current study. The resolution of gene order in the swine genome can be improved by placing these genes and markers on one of the porcine radiation hybrid maps. The order of genes in the human genome will be resolved as the genomic sequence of HSA 4p16 is finished.

The current MARC linkage map has 18 markers in the first 10 cM and 24 markers in approximately the first 15 cM of SSC 8. Seven microsatellite markers are located within the 6.9 cM confidence interval for the QTL. This marker density permits selection of informative markers for most swine populations. The markers selected to be genotyped in the resource population were quite informative, and generally there were over 900 informative meioses (exceptions were SWC31, SB60, and SB73). Five markers were within 1 cM of the peak *F*-ratio; four additional markers extending to the terminal region of SSC 8p and five markers extending toward the centromere of SSC 8p were typed across the entire resource population. Based on the number of highly informative markers typed in a small genomic interval, prediction of breed of origin for chromosomal segments was quite accurate and prediction of founding alleles within each breed is possible.

Further improvements in the estimated location of the QTL will require implementing different statistical models to the data set. The statistical model used in the present study assumes that QTL alleles are fixed for alternate alleles in the founding breeds. Additional genetic markers will not improve the resolution of the QTL under the current statistical model since the markers used accurately predict breed of origin. If the QTL alleles were not fixed for alternate alleles in the parental breeds, then the ability to accurately predict the QTL's position and effect are compromised. Because a significant QTL has been detected under the breed specific statistical model, there is a difference in the average allele contributed by the Meishan breed vs. the White Composite line. A statistical model fitting each of the 40 founding alleles of the resource population would be more powerful and able to evaluate the assumptions under the breed specific statistical model.

The origin of the high ovulating QTL allele comes from the White Composite line, which is contrary to the average breed effects of the Meishan breed (Haley et al., 1995; Young, 1995). The statistical model fitted in this study included an effect for breed composition because gilts were ¼, ½, or ¾ Meishan. The estimate for the regression coefficient indicated that ¾ Meishan:¼ White Composite gilts ovulated 1.8 more ova than ¼ Meishan:¾ White Composite gilts. This difference is smaller than the estimates from Haley et al. (1995) and Young (1995).

The phenomenon of desirable alleles being present in the parental line with the undesirable phenotype is defined as transgressive variation. Transgressive al-

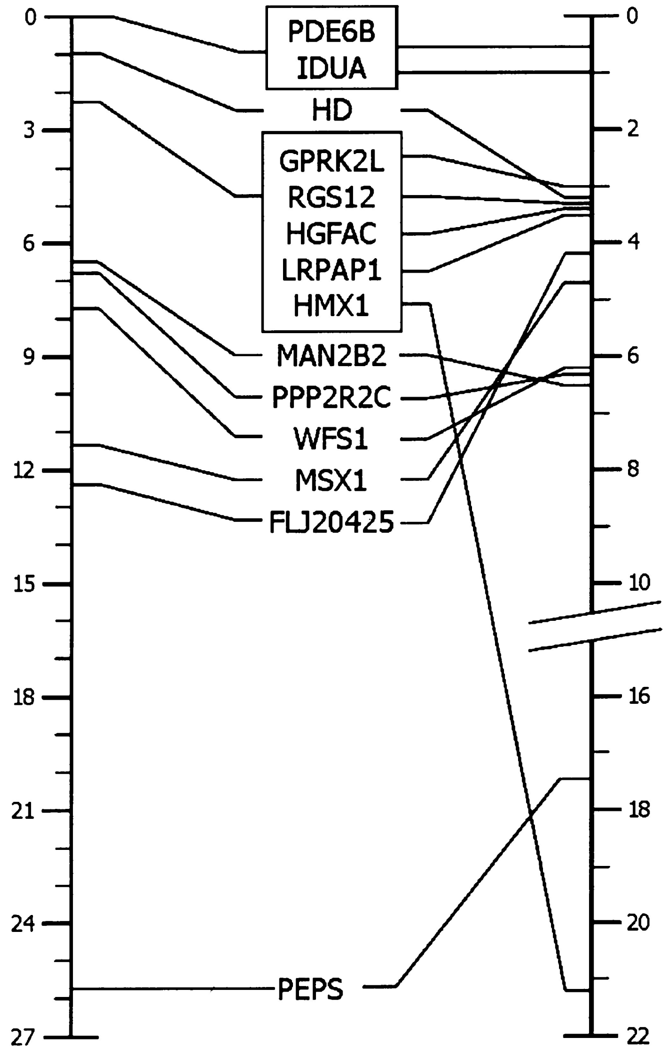

Figure 3. A comparison of the porcine genetic map of chromosome 8 (right side) vs. the physical map or draft genome sequence for human chromosome 4 based on the June 2002 build; displayed at http://genome.ucsc.edu/. Only gene names are presented (described in Table 1); genetic markers related to specific genes are presented in Tables 2 and 3. When no recombination was detected between genes (genes located in boxes), they were listed in the order in which they appear on the human sequence to minimize the number of rearrangements. The porcine genetic map of chromosome 8 is in recombination units (cM) and the human physical map is in millions of base pairs from the p-terminal end of chromosome 4 (4p16.3).

leles have been identified in most comprehensive QTL scans, including a QTL for backfat thickness in swine (Rohrer and Keele, 1998; Bidanel et al., 2001), red color and fruit size in tomatoes (Tanksley and McCouch, 1997), and various other traits. This genomic location has not been detected in other swine populations containing Meishan germplasm for ovulation rate (Wilkie et al., 1999) nor in a scan between selected and control Landrace × Yorkshire pigs (Rathje et al., 1997; Cassady et al., 2001). Whereas this may be due to genetic variation within the Meishan breed, it is also possible that it is due to the occidental breed forming the cross. Of particular interest is the source of the White Composites for this study. Seven of the ten founding White Composite animals were from generations 5 and 6 of a line selected for increased ovulation rate (Leymaster and Bennett, 1994). Therefore, the White Composite animals used in this study may have had superior alleles at this QTL than Yorkshire and Landrace pigs used in other studies.

Selection of positional candidate genes to study is the next phase of this research. Originally, the bone morphogenetic protein receptor 1B was believed to be a good candidate because it was identified as the gene that causes the Booroola effect in sheep (Mulsant et al., 2001; Souza et al., 2001; Wilson et al., 2001), and it resides on HSA 4. However, it maps to SSC 8q2.5 by linkage analysis and is not contained within the confidence interval of the ovulation rate QTL (Kim et al., 2003). Based on the estimated position from this analysis, MAN2B2 is clearly the closest mapped gene to the location of the maximal F-ratio. Furthermore, α-mannosidases are responsible for cleaving mannose residues off proteins before the addition of other saccharide units to form glycoproteins (Kornfeld and Kornfeld, 1985). Follicle-stimulating and luteinizing hormones are two key glycoprotein hormones involved in ovulation. The type and level of glycosylation of these key hormones has been shown to affect activity and clearance rate (Ulloa-Aguirre et al., 1999). Therefore, variation in MAN2B2 could affect the potency of these key reproductive hormones.

Despite its location just outside of the confidence interval, RGS12 could be considered as a positional candidate gene as it is a regulator of G-proteins. Such proteins are important signaling molecules involved in a broad range of cellular regulating activities, such as hormone signaling (Chatterjee and Fisher, 2000). Gladney (2000) determined that RGS12 was expressed at a higher rate in ovarian follicles of gilts that were from a Yorkshire-Landrace line selected for increased ovulation rate (Johnson et al., 1999) than in gilts from the control line. In addition to these genes of known function, there are putative genes (based on sequence analysis and expression of transcripts) in this interval with unknown function that may affect ovulation rate.

Implications

A quantitative trait locus has been detected affecting ovulation rate on the terminal end of chromosome 8p. Additional genetic markers were developed for this region using a directed comparative mapping approach by selecting genes that mapped to HSA 4p16-15. This approach permitted a high-resolution comparative map for SSC 8p2.3 and provided a sufficient number of markers to determine whether this quantitative trait locus is segregating in commercial swine populations. Nine genes were localized to the first 8 cM of the linkage group but only three are within the confidence interval of the quantitative trait locus in this study. The next phase of this research is to utilize the genetic markers in commercial swine populations where ovulation rates have been measured to determine whether this quantitative trait locus is segregating in commercial pigs. In addition, positional candidate genes can now be selected from the human genome sequence located within the first 10 Mb of HSA 4 and evaluated in the founding animals of the U. S. Meat Animal Research Center swine resource population.

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